

## Influence of parity of birth and suckled sows on piglet nasal mucosal colonization with *Haemophilus parasuis*

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**Abstract** — Litters of primiparous and multiparous sows were switched at 12 d and nasally swabbed at 12, 18, and 23 d for detection of *Haemophilus parasuis*. At lactation days 12 and 23, mucosal colonization rates for multiparous and primiparous litters were 0% versus 33% and 26% versus 60%, respectively.

**Résumé** — Effet de la parité des truies biologiques et adoptives sur la colonisation de la muqueuse nasale des porcelets par *Haemophilus parasuis*. Les portées issues de truies primipares et de truies multipares ont été échangées à 12 jours d'âge. Des prélèvements nasaux ont été réalisés à 12, 18 et 23 jours d'âge afin de détecter la présence d'*Haemophilus parasuis*. Au 12<sup>ème</sup> et au 23<sup>ème</sup> jour de lactation, les taux de colonisation de la muqueuse des portées élevées par des truies multipares et des portées élevées par des truies primipares étaient respectivement de 0 % contre 33 % et de 26 % contre 60 %.

(Traduit par les auteurs)

Can Vet J 2016;57:1281–1283

The term “early colonizers” was coined by Pijoan (1) to describe commensal bacteria that colonize the pig's mucosal surfaces during the first 2 wk of life such as *Haemophilus parasuis* which, depending on the serotype, may or may not be pathogenic. Assuming adequate colostrum intake by the piglets, their passive immunity will protect them against these early colonizers, although the degree of protection will progressively decline during lactation and, by about 14 d, the level of protection fails to prevent colonization but maintains protection against disease until the pig's own active immunity develops some weeks later (2). This controlled pathogen exposure allows the development of immunological defenses and so limits the likelihood of disease carriage into the nursery.

The sow's colostrum is an essential source of energy, maternal antibodies, and immune cells and ensures the passive protection of piglets until their own immune system matures (3). An effect of sow parity on colostrum and milk quality and quantity has been described. Specifically, colostrum and milk yields were lower from primiparous than from higher parity

sows (4) and the concentration of IgG in colostrum was lower from primiparous than from multiparous sows (5,6). A potentially reduced passive immunity of piglets born to primiparous sows due to their lower colostrum yield and IgG content may impact the dynamics of mucosal colonization of bacteria such as *H. parasuis*. Indeed, a delayed mucosal *H. parasuis* colonization was observed in piglets from primiparous, compared to those from multiparous sows (7). Possibly, the young primiparous sows did not provide the infectious pressure of older sows and, presumably, a delayed mucosal colonization would also apply to other potential respiratory or enteric pathogens. The net effect would be that pigs are colonized at an older age when the already limited passive protection is even further reduced. The relatively poor immunological status of pigs born to young sows may increase the risk of their carrying pathogens into the nursery. The consequence of this would be a destabilization of nursery health with higher veterinary inputs and slower growth (8). This problem can be countered using parity segregated pig flows but would require sufficient pigs for the separate flows.

The present study was undertaken to determine effects of sow parity on piglet mucosal colonization with *H. parasuis* when entire litters were cross-fostered between 10 and 15 d of age. We hypothesized that pigs born to primiparous sows but nursed by multiparous sows would have earlier mucosal colonization by *H. parasuis* than would pigs born to and remaining with their primiparous sow.

This experiment was approved by the University of Adelaide Animal Ethics Committee. A total of 22 Large White × Landrace sows [11 primiparous (P1) and 11 multiparous (P3+)] and their litters were assigned. Sows and litters were housed in farrowing pens with conventional farrowing crates and received a standard lactation diet [14.1 MJ DE/kg body weight (BW),

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**Table 1.** Effect of piglet birth sow parity and nurse sow parity on piglet nasal mucosal colonization by *Haemophilus parasuis*

Age at swabbing	P1/p1 <sup>a</sup>	P3/p3	P1/p3	P3/p1
Litters <sup>b</sup>				
12 d	4/6	0/5	0/5	1/6
18 d	6/6	2/5	3/5	2/6
23 d	6/6	3/5	3/5	2/6
Piglets				
12 d	15/30 <sup>c</sup>	0/25 <sup>e</sup>	0/25 <sup>e</sup>	5/30 <sup>d</sup>
18 d	23/30 <sup>c</sup>	2/25 <sup>d</sup>	5/25 <sup>d</sup>	6/30 <sup>d</sup>
23 d	27/30 <sup>c</sup>	3/25 <sup>d</sup>	10/25 <sup>d</sup>	9/30 <sup>d</sup>

<sup>a</sup> Uppercase P refers to the nurse sow parity while lowercase p refers to birth parity of the litter (e.g., P1/p3 means a P1 sow nursing p3 piglets after litter transfer); P3 includes parities 3 or older.

<sup>b</sup> Litters where at least 1 of 5 piglets were positive for *H. parasuis*. Means were compared using Fisher's exact test at litter transfer and by logistic regression preweaning. Means followed by different letters (<sup>c,d,e</sup>) differ significantly,  $P < 0.05$ .

16.5% CP, 1.0% total lysine] fed-to-appetite until piglets were weaned at 28 d of age. At  $12.3 \pm 0.3$  d of age entire litters were exchanged between P1 and P3+ sows, with a maximum age difference of 3 d, or were not exchanged, to create 4 treatment groups: 6 P1 sows nursing P1 litters, 5 P3+ sows nursing P3+ litters, 5 P1 sows nursing P3+ litters, 6 P3+ sows nursing P1 litters.

Sow behavior towards the fostered litter was monitored for 1 h after cross-fostering. At the time of litter transfer, 5 randomly selected piglets per litter were ear tagged. These piglets were nasally swabbed at litter transfer and again 6 d and 11 d later for *H. parasuis* culture and polymerase chain reaction (PCR).

In order to identify *H. parasuis*, swabs were initially cultured on Chocolate Agar and Sheep Blood Agar (SBA) plates with *Staphylococcus aureus* streak inoculation (V factor) (9). The plates were incubated at 37°C for 48 h and *H. parasuis* cultures were identified as small halo transparent clusters of colonies around the *S. aureus* stabs. Presumptive *H. parasuis* colonies were transferred onto another SBA plate (with *S. aureus* streak inoculation) and incubated for 48 h at 37°C. If *H. parasuis*-like colonies were identified by sub-culturing, the colonies were subjected to a catalase test and Gram staining. If the isolate was dependent on *S. aureus* for growth, catalase positive, Gram-negative, and grew on chocolate agar, DNA was extracted and *H. parasuis* was confirmed by 16S RNA PCR (9). In addition to culturing, an additional nasal swab was collected from every animal and was subjected to direct DNA extraction to detect the presence of *H. parasuis* by PCR (9). The animal was considered positive for *H. parasuis* if *H. parasuis* was cultured and confirmed by PCR or if the direct *H. parasuis* PCR from the swabs was positive.

The DNA was extracted as per methods described previously (10). In short, 4 to 6 bacterial colonies or freshly obtained nasal swabs were suspended in 1 mL of sterile water and tubes were centrifuged for 1 min at  $10\ 730 \times g$ . Supernatant was removed and 200  $\mu$ L of 6% Chelex was added to the bacterial pellet. Tubes were vortexed for 10 s and incubated at 56°C for 20 min. Tubes were then re-vortexed for 10 s and incubated at 100°C for 8 min. Finally, tubes were centrifuged for 5 min at  $10\ 730 \times g$  and stored at 4°C (10).

To detect the presence of *H. parasuis* 16S RNA, PCR was performed as previously reported (9). The PCR was performed to detect the presence of *H. parasuis* from DNA extracted from nasal swabs and to confirm the detection of *H. parasuis* from culture. The PCR was performed using 2 specific primers: HPS-forward (5' GTG TG AGG AAG GGT GGT GT 3') and HPS-reverse (5' GGC TTC GTC ACC CTC TGT 3') (9). The PCR was performed in a 25- $\mu$ L reaction mixture containing 2  $\mu$ L of extracted DNA, 16.8  $\mu$ L of PCR H<sub>2</sub>O, 5  $\mu$ L of 5 $\times$  PCR Buffer (My *Taq* PCR Buffer, Bioline, Australia), 0.5  $\mu$ L of each primer (50  $\mu$ M) and 0.2  $\mu$ L of My *Taq*<sup>TM</sup> DNA Polymerase (Bioline, Australia). The PCR was carried out for 30 cycles consisting of denaturation for 30 s at 95°C, annealing for 30 s at 59°C and extension for 1 min at 72°C using a thermal cycler. The PCR products were run in agarose gel for 75 min at 120 V. Gels were stained with RedGel and put in a box for 1 h before being photographed.

Data analyses were performed using ASReml 3.0 (VSN International, Hemel, Hempstead, UK). Age and parity effects on proportions of litters and piglets colonized by *H. parasuis* prior to litter swap were examined using Fisher's exact test. Proportions of litters colonized preweaning were examined using a binomial regression with a logit link function.

Patterns of mucosal colonization by *H. parasuis* appeared to be affected by parity of the birth sow, with litters of P1 sows having more piglets colonized at the time of litter transfer and at 18 and 23 d than did those of litters born to P3+ sows ( $P < 0.001$ ).

At litter transfer, litters of P1 sows nursed by P1 sows were 0%, 0%, 20%, 80%, 100%, and 100% colonized. All litters were colonized by 18 d with high colonization rates (Table 1). Final colonization rates were 80%, 80%, 80%, 100%, 100% and 100%.

For P3+ litters nursed by P3+ sows, no piglet was colonized at the time of litter transfer with relatively few pigs becoming colonized during lactation (Table 1). Final colonization rates were 0%, 0%, 20%, 20%, and 20%.

For P3+ litters nursed by P1 sows, no pig was colonized at the time of litter transfer but more became colonized during lactation compared to pigs born to P3+ sows (Table 1). Final colonization rates were 0%, 0%, 40%, 80%, and 80%.

For P1 litters nursed by P3+ sows, 1 litter had 100% colonized at litter transfer with a second litter becoming colonized by 18 d. Final colonization rates were 0%, 0%, 0%, 80%, and 100%.

We observed no aggression of the sow towards the piglets or amongst the piglets. Sow aggression towards fostered piglets, and aggression between piglets, is minimized by completing cross-fostering by 24 h after farrowing. When fostering part litters at 4 to 7 d of age, sow aggression towards piglets was increased and successful suckling bouts decreased (11). We transferred piglets at about 10 d of age without behavioral consequences for the sow or litter. This is likely due to the total absence of the sows own litter and the transfer of entire litters causing less disruption to teat order.

In the present study, no piglets born to P3+ sows were positive for *H. parasuis* at the time of litter transfer. In comparison,

33% of piglets born to P1 sows were positive for *H. parasuis* when transferred at approximately 12 d of age. These data contrast with those of earlier workers who found that at 14 d of age, 53% of piglets derived from multiparous sows yielded a positive culture of *H. parasuis* while none of the piglets derived from primiparous sows were positive (7). The present data indicate that piglets born to older sows are likely to have a delayed colonization by *H. parasuis*, and likely other mucosal commensals, possibly due to an enhanced passive immunity of these piglets. Indeed, it has been shown that *H. parasuis* colonization of piglets was delayed and the heterogeneity of *H. parasuis* strains isolated was reduced when their sows received *H. parasuis* vaccination prepartum (12). The significance of the current findings for nursery pig health is unclear since it has been hypothesized that a low level of *H. parasuis* colonization at weaning could predispose to clinical disease in the nursery (1). However, although speculative, it is possible that the lower colonization levels in piglets from our multiparous sows reflects an improved level of passive protection at nursery entry and that in the absence of a subpopulation of shedding pigs (i.e., primiparous progeny) it is a minor concern. This poses the question for nursery pig health and mechanisms involved in parity segregation; are progeny of multiparous sows infecting progeny of primiparous sows, or vice versa?

### Acknowledgment

This trial was funded by the South Australia Pig Industry Advisory Group. CVJ

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